J. Physiol. (1978), **275**, *pp.* 439–447
With 4 *text-figures*
Printed in Great Britain

AUTONOMIC THERMOREGULATION AFTER INTERMITTENT COOLING OF THE SPINAL CORD AND COLD EXPOSURE IN THE RAT

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(*Received 12 September 1977*)

SUMMARY

1. O_2 consumption, rectal and several skin temperatures were studied, at various ambient temperatures, in unanaesthetized rats that had been thermally stressed for an average of 290 h either by prolonged and intermittent cooling of the spinal cord or by prolonged and intermittent exposure to an ambient temperature which induced the same increase in O_2 consumption as did the thermal stimulation of the spinal cord.

2. At all the test ambient temperatures, both groups of thermally stressed animals maintained a metabolic level higher than that of the controls. In the animals previously exposed to cold the extent by which the metabolic rate was greater than that of the control animals was independent of ambient temperature; in those previously subjected to cooling of the spinal cord, however, it increased as the ambient temperature was lowered. Rectal and average skin temperatures were essentially unaffected by the treatments.

3. It is concluded that prolonged and intermittent cooling of the spinal cord increases the gain of the temperature control system, whereas prolonged and intermittent cold exposure has no effect on it, and that these forms of thermal stimulation are therefore not equivalent.

INTRODUCTION

Prolonged cold exposure of the rat under controlled laboratory conditions induces a number of adaptative changes that improve survival during severe cold stress. These changes include increases in the capacity for non-shivering thermogenesis (Sellers, Scott & Thomas, 1954; Cottle & Carlson, 1956) and in cutaneous vascularization, the latter change leading to higher subpelage skin temperatures (Héroux, 1959). Since pelage insulation in the rat is not improved by artificial cold acclimation (Héroux, Depocas & Hart, 1959), an increased flow of heat to the environment can be assumed, and this is consistent with the observation that cold-acclimated rats maintain higher metabolic rates than non-acclimated controls (Depocas, Hart & Héroux, 1957; Héroux *et al.* 1959). Cold appears to be the adequate stimulus for these adaptative changes (Hart, 1971) but its site and mode of action are not yet known. The effect of cold could be on the thermal sensors, and the induction of cold-acclimation be due to the prolonged activation of the neural structures or the effectors concerned with cold defence; on the other hand, the induction of cold-acclimation could be due, at least partly, to a direct effect of cold on peripheral tissues. If the former possibility is correct, it should be possible to induce cold acclimation by local cooling of the central thermoreceptive areas, whereas, if the latter is correct, cold acclimation should be achieved only when peripheral tissues are also cooled.

Previous work in this field (Banet & Hensel, 1976*a*, *b*; Cormarèche-Leydier, Banet, Hensel & Cabanac, 1977) has not provided conclusive results. Prolonged but intermittent cooling of either the preoptic area or the spinal cord induced an increase in the capacity for non-shivering thermogenesis in the rat, and also appeared to facilitate the flow of heat to the environment. These effects suggest that the animals might have become acclimated to cold. On the other hand, this core cooling failed to increase the resistance to severe cold stress, and it increased the operative thermoregulatory behaviour, an increase that is at variance with the reported decrease in thermoregulatory behaviour of cold acclimated rats (Laties & Weiss, 1960).

This failure to induce increased cold resistance by local cooling of the central thermoreceptive areas cannot, however, be interpreted as indicating that cooling of peripheral tissues is necessary, since the behavioural data indicated that longer cooling times may be required to induce full acclimation (Cormarèche-Leydier *et al.* 1977). In the present experiments we compare some autonomic thermoregulatory responses in rats whose cold defense mechanisms had been intermittently stimulated by local cooling of the spinal cord with those of rats in which the same degree of thermal stimulation, as evaluated by the metabolic response, had been obtained by intermittent cold exposure. The results show that both forms of thermal stimulation are not equivalent.

METHODS

A group of white male rats, housed at 23 °C with artificial illumination and a day–night cycle of 12 h, was anaesthetized with 45 mg nembutal/kg and spinal cord thermodes were implanted as previously described (Banet & Hensel, 1976*b*). The animals were then trained to rest quietly in a small chamber and those that apparently had not suffered motor impairment were randomly distributed into three groups: ten in the spinal cord cooled group, eight in the intermittently cold exposed and four in the control group. The last group was supplemented with three more animals without thermodes.

One week after the operation O_2 consumption was measured in the animals of the three groups by an open circuit method (Banet & Hensel, 1976*c*). The rats, partially restrained by taping the tail to a holder, were placed at the selected ambient temperature at least 1 h before the beginning of the experiment; O_2 consumption was then measured at 5 min intervals for 30 min. In the animals of the control group O_2 consumption was measured at normal room temperature (20–22 °C), as it was also in those of the spinal cord cooled group but while the spinal thermodes were perfused with water at 10 °C; in those of the intermittently cold exposed group it was measured, always without central thermal stimulation, at various ambient temperatures. At an ambient temperature of 0 °C (Fig. 1), the animals of the intermittently cold exposed group maintained a level of oxygen consumption similar to that of the spinal cord cooled ones when their thermodes were perfused with water at 10 °C during exposure to normal room temperature.

The day after these measurements were completed, the animals of the cold exposed group were placed at an ambient temperature of 0 °C, while those of the spinal cord cooled group were kept at normal room temperature and thermally stimulated by perfusing the thermodes with water at 10 °C. After an average of 10 h of continuous thermal stimulation, cooling was discontinued and both groups of animals were returned to their home cages at 23 °C, where food and water were available *ad lib.* This procedure was repeated 6 days a week until the animals had been thermally stimulated for an average of 290 h. In the weeks between the implantation and the experiments that followed, however, several animals were lost because the screws holding the thermodes became loose, or the thermodes were otherwise damaged, so that the experiments could be completed with only six animals of each group.

At the end of this cold treatment, all animals, unrestrained and without artificial manipulation of their spinal cord temperatures, were exposed to ambient temperatures of -5, $+5$, 15, 25 and 30 °C. After 75 min of exposure, O_2 consumption was measured over a period of 15 min. Immediately afterwards, and while still exposed to the selected ambient temperature, rectal temperature was measured by inserting a thermocouple about 50 mm past the anal sphincter, while several cutaneous temperatures were measured by gently applying a thermocouple to the surface of the skin, the fur being parted whenever necessary. Cutaneous temperatures were measured in the midline of the dorsal surfaces of the head, neck, thorax and abdomen; in the lateral surface of the thorax at the level of the heart; in the right fore- and hindleg at the level of the knee; in the internal surface of the ear halfway between the base and the tip; in the tail about 20 mm from its base; and in the dorsal surface of the right hindfoot. Average cutaneous temperatures were calculated as the arithmetic mean of the temperatures of the areas concerned.

The above measurements took 1 week to complete. During this time both groups of experimental animals could be cold stressed for an average of only 5 h per day, after the above measurements had been completed. Thus, some de-acclimation could have occurred. To reactivate the acclimation mechanisms, therefore, both experimental groups were again submitted to the cold treatment for 10 h per day until the total cold treatment averaged 400 h. All animals were then exposed to -30 °C and rectal temperature was measured every hour.

RESULTS

At normal room temperature, acute cooling of the spinal cord with water at 10 °C induced an increase in O_2 consumption of almost 100 %. This increase was similar to that elicited by acute exposure to an ambient temperature of 0 °C (Fig. 1). Since the quantitative response to acute cooling of the spinal cord is not significantly affected by the length of cooling (Banet & Hensel, 1976*b*) it would seem that both groups of animals were submitted to equivalent thermal stresses at least at the beginning of the cold treatment, i.e. before the adaptative changes described below took place.

At the beginning of the experiments, body weight averaged 330 ± 10 g (standard error) and was similar in the three groups of animals. After 290 h of thermal stress, however, average body weights for the control, cold exposed and spinal cord cooled groups were 393 ± 21, 368 ± 13 and 341 ± 15 g respectively. To correct for these differences in body weight, O_2 consumption is expressed as a function of body weight using the standard interspecies exponent of 0·73 (Brody, 1945). In the three groups of animals, O_2 consumption at 25 °C was similar to that at 30 °C and the average of both measurements was, therefore, taken as resting O_2 consumption at thermoneutrality. The cold exposed animals had a resting O_2 consumption significantly higher ($P < 0{\cdot}01$) than that of the controls, the values being $17{\cdot}7 \pm 0{\cdot}7$ and $15{\cdot}3 \pm 0{\cdot}4$ ml./min.kg$^{0{\cdot}73}$, respectively. The spinal cord cooled animals had also a higher metabolic rate, $16{\cdot}4 \pm 1{\cdot}6$ ml./min.kg$^{0{\cdot}73}$, but the difference was not significant.

Fig. 2 shows the average O_2 consumption of each of the three groups of animals during exposure to various ambient temperatures, and the lines, calculated by the method of least squares, that best fit these data. The slopes of these lines for the control, cold exposed and spinal cord cooled groups were $-0{\cdot}61$, $-0{\cdot}62$ and $-0{\cdot}81$ ml./min.kg$^{0{\cdot}73}$.°C respectively, while the intercepts, were 30·7, 33·0 and 36·4 ml./min.kg$^{0{\cdot}73}$ respectively. The differences between these parameters were compared by analysis of co-variance. The slope of the correlation line of the spinal cord cooled animals differed significantly from those of both the control and the cold exposed animals ($P < 0{\cdot}01$). On the other hand, the slopes of the control and cold exposed animals did not differ significantly but the intercepts did ($P < 0{\cdot}005$).

Average rectal temperature, and average cutaneous temperatures of furred and unfurred areas at various ambient temperatures are shown in Fig. 3. Neither the slopes nor the intercepts of the correlation lines differed significantly. The only significant temperature differences were observed in the dorsal surface of the neck in the proximity of the cervical brown adipose tissue. In the control animals, subpelage

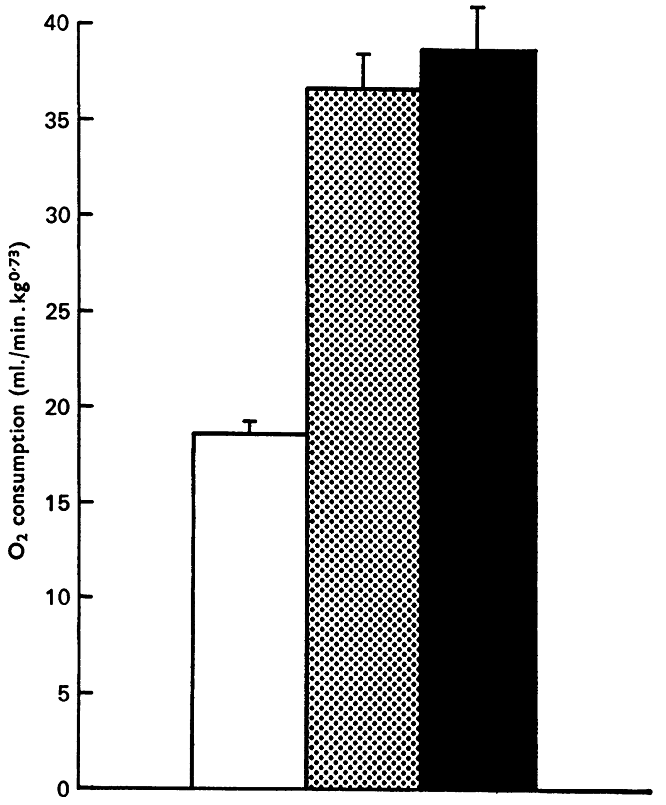

Fig. 1. O_2 consumption in control animals at normal room temperature (open area), in spinal cord cooled animals during thermal stimulation of the spinal cord at normal room temperature (dotted area) and in cold exposed animals at an ambient temperature of 0 °C (solid area). The vertical lines are standard errors.

temperature in this area fell with ambient temperature, but less than in other furred areas. In the cold exposed and spinal cord cooled animals, however, the temperature in this area remained constant at about 35 °C at all ambient temperatures between 25 and −5 °C.

Finally, after an average of 400 h of thermal stress, equivalent to almost 17 days of continuous cold exposure, the resistance to cold was estimated by measuring the rate of drop of rectal temperature during exposure to an ambient temperature of −30 °C. Fig. 4 shows that both thermal treatments had little or no effect on the resistance to severe cold stress.

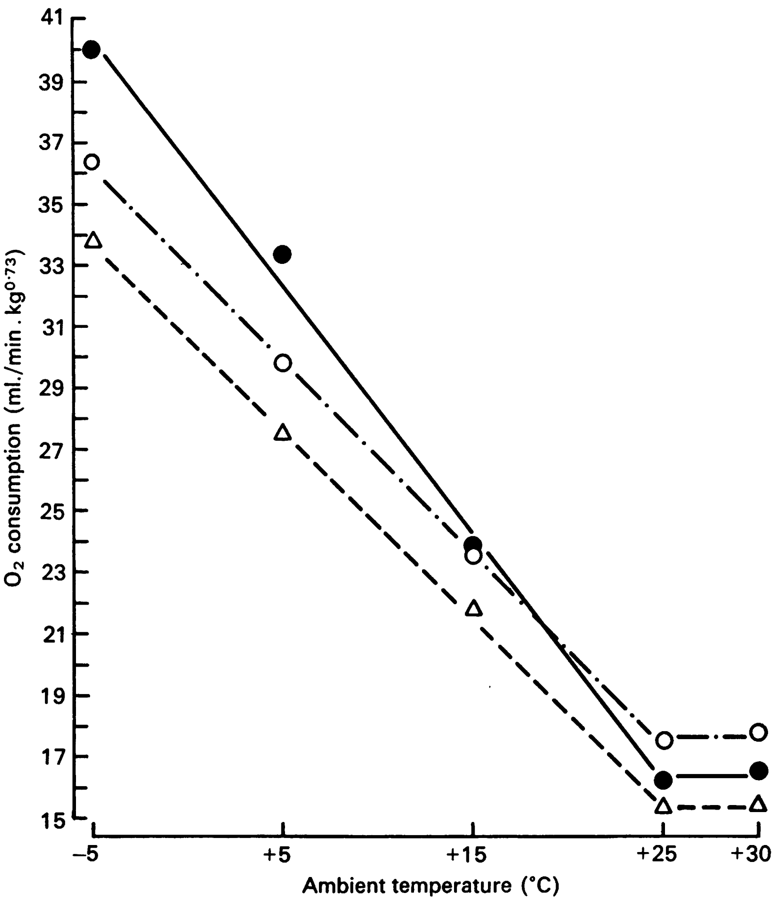

Fig. 2. Average oxygen consumption at various ambient temperatures in spinal cord cooled (●), cold exposed (○) and control (△) animals.

DISCUSSION

In previous experiments it has been shown that intermittent thermal stimulation of either the preoptic area or the spinal cord induces an increase in the capacity for non-shivering thermogenesis and leads to changes that appear to facilitate the flow of heat to the environment (Banet & Hensel, 1976*a*, *b*; Cormarèche-Leydier *et al.* 1977). The present work shows that both intermittent cold exposure and intermittent cooling of the spinal cord induced significant changes in heat production, though neither of the treatments induced a change in the lower critical temperature. Intermittent cold exposure increased heat production above the level of the control animals and this increase was more or less similar at all ambient temperatures. On the other hand, intermittent cooling of the spinal cord had little or no effect on heat production at thermoneutral temperatures; at cold ambient temperatures, however, these animals maintained a level of heat production higher than that of the controls and the difference was greater the lower the ambient temperature. At any ambient temperature, the three groups of animals maintained similar core temperatures and the higher rate of heat production of both groups of experimental animals must therefore have reflected an increase in the rate of heat loss due to an increase in total (core to ambient) conductance. The increased rate of heat production of the cold exposed animals, similar to that of cold-acclimated rats (Depocas *et al.* 1957; Héroux *et al.* 1959), could be accounted for by an increase in core to skin conductance (Héroux,

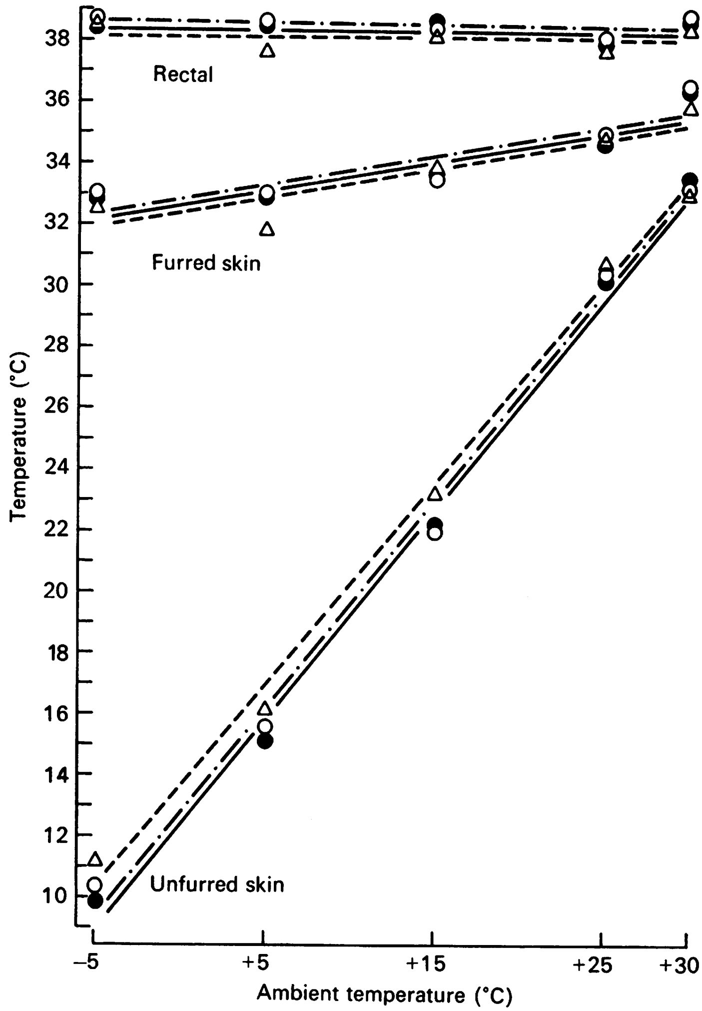

Fig. 3. Average rectal and mean cutaneous temperatures in furred and unfurred areas at various ambient temperatures in spinal cord cooled (● —— ●), cold exposed (○ – · – ○) and control (△ ---- △) animals.

1959), since they had somewhat higher subpelage temperatures than the control animals. The same mechanism might have contributed to the increase in heat production of the spinal cord cooled animals, but this increase in internal conductance cannot provide a satisfactory explanation because the differences in heat production between the spinal cord cooled and the cold exposed animals were qualitative rather than quantitative. Furthermore, the thermoregulatory behaviour of the spinal cord cooled animals shows that their increased rate of heat transfer cannot be caused by an increase in pelage conductance (Cormarèche-Leydier *et al.* 1977); thus, it has to be due to either an increase in evaporative heat loss or to the failure of these animals to decrease behaviourally the effective body surface during acute cold exposure. No changes in posture, however, have been observed.

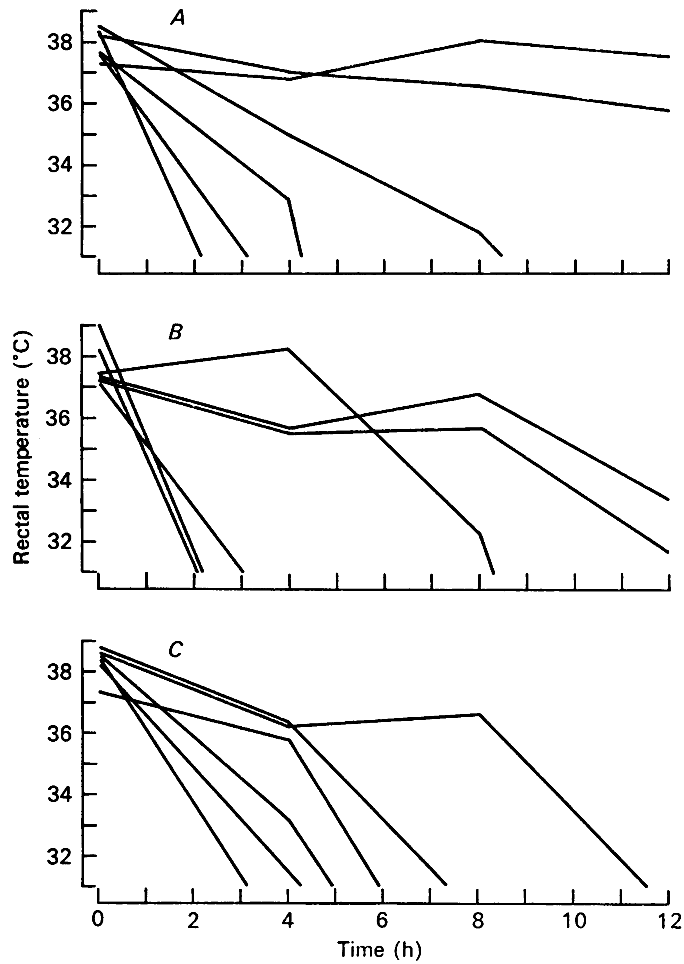

Fig. 4. Changes in rectal temperature during exposure to an ambient temperature of − 30 °C in, (*A*) spinal cord cooled, (*B*) cold exposed, (*C*) control animals.

There is general agreement that the temperature regulation centre processes thermal information from various widely distributed thermoreceptive areas, though the contribution of each thermoreceptive area to the control of body temperature is not yet clear. In the rat, both rectal (Depocas *et al.* 1957) and hypothalamic temperatures (Lomax, Malveaux & Smith, 1964) remain relatively constant during cold exposure, whereas the temperature of the spinal cord is not likely to fall significantly because this area is warmed by the heat produced in the cervical and interscapular brown adipose tissue (Brück & Wünnenberg, 1970). Furthermore, clamping the temperature of the spinal cord to about body temperature did not affect the thermoregulatory responses induced by exposure to mild cold stress (Banet & Hensel, 1976*c*). It would therefore appear that only the cutaneous thermoreceptors could provide an adequate signal for the control of body temperature during acute cold exposure. Since the animals of the three groups maintained similar average cutaneous temperatures at any ambient temperature, the metabolism–temperature curves of Fig. 2 would then reflect the gain of the temperature control system. Thus, intermittent cold exposure does not seem to have changed the characteristics of the control system and the increased O_2 consumption at any ambient temperature appears only to reflect the higher resting metabolic rate. On the other hand, intermittent cooling of the spinal cord induced what appears to be a genuine change in gain since, if as a result of the chronic central cold stimulation the temperature in another central thermoreceptive area had decreased, this additional cold stimulation would have induced a change in threshold rather than in gain (Banet & Hensel, 1976*c*).

A surprising result of the present experiments was to find that intermittent cold exposure did not induce an increase in the resistance to severe cold stress. The increase in O_2 consumption was similar to that found in cold-acclimated rats (Depocas *et al.* 1957; Héroux *et al.* 1959), and the increase in subpelage temperature in the dorsal surface of the neck (see Results) suggests that they had a capacity for non-shivering thermogenesis higher than that of the control animals. On the other hand, the increase in cutaneous temperatures was not as high as might have been expected (Héroux, 1959); whether these lower cutaneous temperatures are characteristic of intermittent as opposed to continuous cold exposure or the result of the failure to induce full acclimation, is not known. Intermittent cold exposure induces a lower acclimation effect than continuous exposure and the final acclimation effect, which depends on the relative duration of the cold interval, is reached within 32 days of the treatment (Andjus, Rizvanoli & Ristanović, 1971). The intermittently cold exposed animals should then have reached their final acclimation state, but the cold treatment probably induced only a moderate increase in cold resistance because the cold interval of the treatment was relatively short. Thus, it may be possible that the standard thermal stress was too severe to reveal the increase in cold resistance. From this point of view these results are inconclusive for they do not determine whether or not local cooling of a central thermoreceptive area can induce full acclimation. On the other hand, they clearly show that the long term effect of intermittent cold exposure on the control of heat production is not the same as that of intermittent local cooling of a central thermoreceptive area.

This work was supported by the Sonderforschungsbereich 122 (Adaptation and Rehabilitation) of the Deutsche Forschungsgemeinschaft.

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